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# Nucleotide Regulation of a Calcium-activated Cation Channel in the Rat Insulinoma Cell Line, CRI-G1

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**Abstract.** The nucleotide regulation of a calcium-activated nonselective cation (Ca-NS$^+$) channel has been investigated in the rat insulinoma cell line CRI-G1. The activity of the channel is reduced by both AMP and ADP (1–100 μM) in a concentration-dependent manner, with AMP being more potent than ADP. At lower concentrations (0.1–5 μM), both ADP and AMP activate the channel in some patches. Examination of the nucleotide specificity of channel inhibition indicates a high selectivity for AMP over the other nucleotides tested with a rank order of potency of AMP > UMP > CMP ≥ GMP. Cyclic nucleotides also modulate channel activity in a complex, concentration-dependent way. Cyclic AMP exhibits a dual effect, predominantly increasing channel activity at low concentrations (0.1–10 μM) and reducing it at higher concentrations (100 μM and 1 mM). Specificity studies indicate that the cyclic nucleotide site mediating inhibition of channel activity exhibits a strong preference for cyclic AMP over cyclic GMP, with cyclic UMP being almost equipotent with cyclic AMP. Cyclic IMP and cyclic CMP are not active at this site. The cyclic nucleotide site mediating activation of the channel shows much less nucleotide specificity than the inhibitory site, with cyclic AMP, cyclic GMP and cyclic IMP being almost equally active.

**Key words:** Rat insulinoma cell line, CRI-G1 — Nucleotide regulation — Calcium-activated nonselective cation channel — Patch clamp

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## Introduction

Calcium-activated nonselective cation (Ca-NS$^+$) channels are found in a wide range of vertebrate and invertebrate cells where they are likely to play important roles in excitation-secretion coupling and in the production of slow depolarizing waves which underlie action potential firing patterns (Partridge & Swandulla, 1988). There has also been a report of this channel in the endosperm plasma membrane of higher plants (Stoeckel & Takeda, 1989). The apparently ubiquitous nature of Ca-NS$^+$ channels has led to the suggestion that they may represent the archetypal cation channel from which the more specialized channels for $Na^+$, $K^+$ and $Ca^{2+}$ have evolved (Hille, 1984).

Ca-NS$^+$ currents were first described in cardiac Purkinje fibers (Kass et al., 1978) and subsequent work using single channel recording techniques demonstrated the presence of Ca-NS$^+$ channels in rat cultured cardiac ventricular cells (Colquhoun et al., 1981). Since then, similar channels have been found in a wide variety of tissues (*see* Partridge & Swandulla, 1988; Swandulla & Partridge, 1990, for reviews). In general, Ca-NS$^+$ channels have a single channel conductance of between 20 and 35 pS and show little selectivity between $Na^+$ and $K^+$ ions, but exclude anions. They all exhibit a largely linear current-voltage relationship, although the degree of voltage dependence of their open state probability varies from those that show a strong voltage dependence, such as in Schwann cells (Bevan, Gray & Ritchie, 1984), to those that show no obvious voltage dependence, such as in cardiac ventricular myocytes (Colquhoun et al., 1981). Calcium concentrations in the micromolar range are generally required for channel activation in excised patches, with the Ca-NS$^+$ channel in rat Schwann cells being exceptional in re-

quiring >0.1 mM $Ca^{2+}$ for activation (Bevan et al., 1984). Maruyama and Petersen (1982, 1984) noted that the Ca-$NS^+$ channels from pancreatic acinar cells were far more sensitive to $Ca^{2+}$ when the patches were still attached to the cell, and that this sensitivity rapidly declined following excision, suggesting that some endogenous modulators may regulate the channel's sensitivity to $Ca^{2+}$ and prevent it from desensitizing.

Ca-$NS^+$ channels are also present in the plasma membrane of the insulin-secreting cell line, CRI-G1 (Carrington et al., 1986). This channel has a single channel conductance (25 pS) and ion selectivity properties similar to those described previously, but in terms of calcium sensitivity the channel more closely resembles that found in cultured Schwann cells since $Ca^{2+}$ ion concentrations >0.1 mM are required for a significant degree of activation (Sturgess et al., 1986*b;* Sturgess, Hales & Ashford, 1987). Furthermore, the open state probability of this Ca-$NS^+$ channel has also been shown to be reduced in a concentration-dependent way by application of ATP, or the nonhydrolyzable analogue, AMPPNP, to the cytoplasmic surface of the membrane (Sturgess et al., 1986*b,* 1987). Preliminary data also suggest that the channel is sensitive to a range of other adenine derivatives (Sturgess et al., 1986*b*).

The present report details the specificity and sensitivity of the Ca-$NS^+$ channels in CRI-G1 cells to a variety of nucleotides. In addition, because a wide variety of ion channels are now known to be directly gated by second messengers, such as cyclic nucleotides (*see* Hockberger & Swandulla, 1987; Swandulla & Partridge, 1990; Kaupp, 1991), we have also examined the sensitivity of the Ca-$NS^+$ channels in the CRI-G1 cells to a range of these nucleotides. The results are discussed in terms of the regulatory properties of nucleotide derivatives on Ca-$NS^+$ channels and the possible physiological significance of such modulation. A preliminary account of some of these data has been published (Reale, Hales & Ashford, 1992).

## Materials and Methods

### Cell Culture

Cells of the rat pancreatic islet cell line, CRI-G1, were used. These were grown in Dulbecco's modified Eagle's medium at 37°C in a humidified atmosphere of 95% air and 5% $CO_2$ and passaged at weekly intervals (Carrington et al., 1986). For the patch clamp studies, cells of 2–6 days-old inclusive were used. After this time they become unsuitable for experimentation because of their high density.

### Recording and Analysis

In all experiments, the inside-out configuration of isolated membrane patches (Hamill et al., 1981) was used. The recording pipettes used had resistances of 8–10 MΩ. Single channel currents were recorded using a Dagan 8900 patch clamp amplifier, coupled to an 8930 probe (Dagan, Minneapolis, MN). Seal formation was monitored using a digital oscilloscope (Gould 1421) and currents were stored on video cassettes (VHS video cassette recorder, SLV-201, Sony; digital pulse code modulation, PCM-701ES). Recorded data from the experiments were played back into a Gould 3000 chart recorder which filtered the signals at 0.14 kHz. Outward currents (defined as the current flowing from the intra- to extracellular side of the membrane) are indicated as upward deflections of the trace.

Single channel current analysis was determined off-line by use of a program that incorporates a 50% threshold crossing parameter to detect events (Dempster, 1988) and run on an Elonex PC286C-100 microcomputer. Data segments between 1–4 min were replayed into the computer at the recorded speed, filtered at 600 Hz (−3 dB) using an 8-pole Bessel filter and digitized at a frequency of 3.3 kHz using a Data Translation 2801A interface. The average channel activity ($N_f \cdot P_o$) where $N_f$ is the number of functional channels in the patch and $P_o$ is the open state probability, was determined by measuring the total time spent at each unitary current level and expressed as a percentage of the total time recorded (Kozlowski, Hales & Ashford, 1989; Kozlowski & Ashford, 1990). Concentration-effect curves for the inhibitory actions of nucleotides were fitted to the equation

$$y = (a - d)/[1 + (x/c)b] + d \quad (1)$$

where $a$ and $d$ represent the maximum and minimum values, $c$ is the half-maximal inhibitory concentration and $b$ is the slope parameter (Hill coefficient), according to a Marquandt-Levenberg least squares method (SigmaPlot V.4.1). Experimental values are given as mean ± SEM; $n$ denotes the number of results. Statistical comparisons between data sets were made using a Student's $t$-test.

### Solutions

The ionic composition of the solution in the patch pipette was (mM) 135 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$ and 10 HEPES, titrated to pH 7.2 with NaOH. The bathing solution consisted of (mM): 140 KCl, 1 $MgCl_2$, 0.9 $CaCl_2$, 1 EGTA and 10 HEPES, titrated to pH 7.2 with KOH, giving a final free calcium concentration of 1 μM. Because a high [$Ca^{2+}$] is needed to activate the nonselective cation channel (Ca-$NS^+$), the bathing solution was replaced with a solution containing (mM): 140 KCl, 1 $MgCl_2$, 1 or 0.5 or 0.2 $CaCl_2$ and 10 HEPES titrated to pH 7.2 with KOH on formation of an inside-out patch. The extent of the chelation of calcium and magnesium ions by the nucleotides used was calculated by the "METLIG" program (P. England and R. Denton, University of Bristol) and was found to be negligible in the experiments described for all nucleotides up to a concentration of 1 mM.

Drugs were applied to membrane patches by superfusing the bath, using a gravity feed system, at a rate of approximately 0.5 ml/sec, which allowed a complete solution exchange within 45 sec. All experiments were performed at room temperature, 22–25°C. The reagents used were obtained from Sigma Chemical (Poole, Dorset, UK).

## Results

### Inhibition of Ca-$NS^+$ Activity by Adenine Nucleotides

Single channel recordings were obtained from inside-out patches of CRI-G1 cells, exposed to an asymmetrical

distribution of cations across the membrane (i.e., $Na^+$-rich in the electrode and $K^+$-rich bathing the internal face of the excised membranes) and with high concentrations of calcium (0.1–1.0 mM) in the bathing solution. Such high concentrations of calcium are required to produce a significant opening of the Ca-$NS^+$ channel (Sturgess et al., 1987). In most experiments in the present study, 0.5 mM calcium was used in the bathing medium, but in some experiments with AMP and cyclic AMP, where an activation of channel activity was expected, a lower calcium concentration (0.2 mM) was used to maximize the potential for the observation of an increase in channel activity. In all experiments, membrane patches were held at a membrane potential of −45 mV. Under these experimental conditions, the 25 pS Ca-$NS^+$ channel was the predominant ionic channel (Sturgess et al., 1987) with an average of 3.16 ± 0.17 (mean ± SEM) ($n$ = 51) channels per patch. The activity of the $K_{ATP}$ channels present in the patches was largely inhibited by the high internal concentrations of calcium used in these studies (Sturgess et al., 1987) and by the process of rundown (Kozlowski & Ashford, 1990). Patches containing large conductance calcium-activated $K^+$ channel activity (Sturgess et al., 1986*a*) were discarded.

Figure 1 shows typical single channel activity and illustrates the sensitivity of the Ca-$NS^+$ channel to AMP. Exposure of the internal face of the membrane patch to AMP (Fig. 1*A,B*) strongly inhibited channel activity with no change in the unitary channel current amplitude; 10 μM AMP reduced $N_f \cdot P_o$ from 0.46 to 0.05 and 100 μM AMP caused complete inhibition. Channel activity recovered completely when AMP was washed away from the internal face of the membrane. Similar results were obtained on application of ADP to the isolated patch. This is illustrated in Fig. 2 where application of 10 μM ADP reduced $N_f \cdot P_o$ from 0.86 to 0.38 and 100 μM from 1.32 to 0.004. Channel activity recovered completely on removal of the ADP from the patch. The decrease in the activity of the Ca-$NS^+$ channel induced by AMP and ADP could be due to an alteration in the average open-state probability ($P_o$) of the channel and/or in the number of functional channels ($N_f$) observed in a single patch. For example, 10 μM AMP (Fig. 1*A*) and ADP (Fig. 2*B*) reduced the number of active channels from three to two and from two to one, respectively. Unfortunately, a patch with only one functional channel was never obtained, so binomial analysis was performed on the average currents recorded from inside-out patches to try to determine which of these parameters were changing in the presence of the nucleotides (Kozlowski et al., 1989). The results of this study (*not shown*) indicate that both AMP and ADP (10 μM) induce a decrease in both parameters of channel activity, $P_o$ and $N_f$ ($n$ = 3 for each nucleotide). In general, the reduction in $N_f$ was reversible on washout of nucleotide although, on some occasions, the number of channels remained reduced (Figs. 1*A*, 2*A*) and there was a compensating increase in the $P_o$ of the remaining active channels which resulted in little overall change in $N_f \cdot P_o$ compared to control. On the occasions when all channels returned to activity on washout of the nucleotide there was some "refreshment" such that channel activity was transiently higher with respect to the control value. These actions of the adenine nucleotides were not investigated further.

Inhibition of Ca-$NS^+$ channel activity by ADP and AMP was clearly concentration dependent. This is illustrated in Fig. 3 where the activity of the channel in the presence of the nucleotide ($N_f \cdot P_{o\ test}$) is plotted relative to the control activity prior to the addition of the nucleotide ($N_f \cdot P_{o\ control}$) for patches exhibiting inhibition only (*see below*). The calculated half-maximal inhibitory concentrations for AMP and ADP were 0.44 and 3.4 μM with Hill coefficients of 0.96 and 1.47, respectively. For comparison, the concentration-inhibition curve for ATP (half-maximal inhibitory concentration, 8 μM) is also shown using data from Sturgess et al., 1987).

## Activation of Ca-$NS^+$ Channels by Adenine Nucleotides

In some patches AMP (6 out of 26 tested) and ADP (6 out of 20 tested), at concentrations between 0.1 and 5 μM, increased Ca-$NS^+$ activity. Figure 4*A* illustrates this effect for a patch in which application of 0.1 μM AMP increased channel activity from 0.06 to 1.61. There was no obvious concentration dependence observed for this activation, and the mean increase in activity induced by 0.1–5 μM AMP was 1.77 ± 0.32 ($n$ = 6). Similarly, ADP (0.1–5 μM) also increased Ca-$NS^+$ activity. Figure 4*B* illustrates the effect of 0.1 μM ADP applied to an inside-out patch; channel activity increased from 1.45 to 1.93. ADP at this concentration range produced a mean increase in Ca-$NS^+$ channel activity of 2.04 ± 0.50 ($n$ = 6) over control. Both nucleotides increased $P_o$ and $N_f$ as confirmed by binomial analysis (*not shown*). These effects of AMP and ADP were also reversible on removal of the nucleotide, although in some cases $P_o$ remained increased suggesting that some refreshment of activity had occurred. The increase in Ca-$NS^+$ channel activity was maintained during the period (up to 5 min) in which the internal face of the membrane was exposed to nucleotide. In all isolated patches which displayed Ca-$NS^+$ channel activation in response to low concentrations of AMP or ADP, higher concentrations of nucleotide (≥10 μM) caused inhibition. The reason why some membrane patches exhibit an increase and others a decrease in Ca-$NS^+$ channel activity at the same concentration is not known.

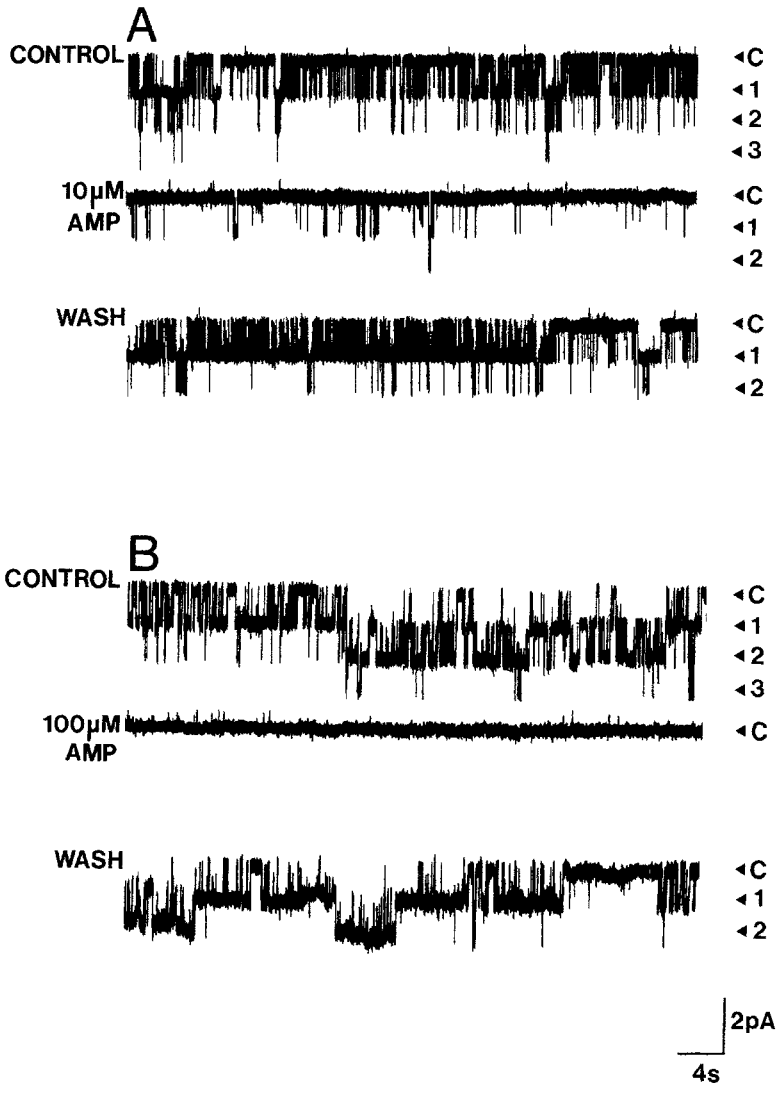


**Fig. 1.** Single channel current records showing the inhibitory effects of two different concentrations of AMP, 10 μM (*A*) and 100 μM (*B*) in two separate patches. Ca-NS$^+$ channel currents were recorded from excised inside-out patches held at a membrane potential of −45 mV in the presence of 0.5 mM $Ca^{2+}$. Single channel openings are denoted by downward deflections (inward currents). The values for $N_f \cdot P_o$ are as follows: (*A*) Control, 0.456; 10 μM AMP, 0.046; Wash, 0.586; (*B*) Control, 0.891; 100 μM AMP, 0; Wash, 0.697. In this figure, and all subsequent figures, the current levels are indicated on the right (*c* = closed; *1* = one channel open; *2* = two channels simultaneously open, etc.)

### Nucleotide Specificity

Since the Ca-NS$^+$ channel was more sensitive to AMP than the other adenine nucleotides tested (*see above and* Sturgess et al., 1986*b*), the nucleotide specificity of the channel was investigated by comparing the relative potencies of a range of monophosphorylated nucleotides.

In contrast to AMP, UMP, CMP and GMP were much less effective at reducing channel activity. They caused incomplete closure of the channel even at concentrations as high as 1 mM (Table 1). All three nucleotides inhibited channel activity by reducing $P_o$ and $N_f$, effects which were reversed on washing (*data not shown*). The inhibitory effects of the non-adenosine-based nucleotides (0.1–1.0 mM) were significantly less than those of 0.1 mM AMP ($P < 0.01$). The results indicate that the Ca-NS$^+$ channel shows a high selectivity for inhibition by AMP over the other nucleotides tested, with a rank order of potency of AMP > UMP > CMP ≥ GMP. There was no activation induced by low concentrations (0.1 and 1.0 μM) of the non-adenosine monophosphorylated nucleotides ($n = 7$).

### Actions of Cyclic Nucleotides on Ca-NS$^+$ Channels

Application of cyclic AMP to the internal face of excised patches also induced dual effects on Ca-NS$^+$ channel activity. At low concentrations (0.1 and 1.0 μM) cyclic AMP caused either activation or a small reduction of channel activity. Figure 5*A* shows that 1 μM cyclic AMP induced an increase in channel activity which was maintained for as long as the patch was exposed to the nucleotide and was reversible on washout of the cyclic AMP. Activation of channel activity by 0.1 and 1.0 μM cyclic AMP was observed in 14 out of 20 patches analyzed. In the other six patches, a small

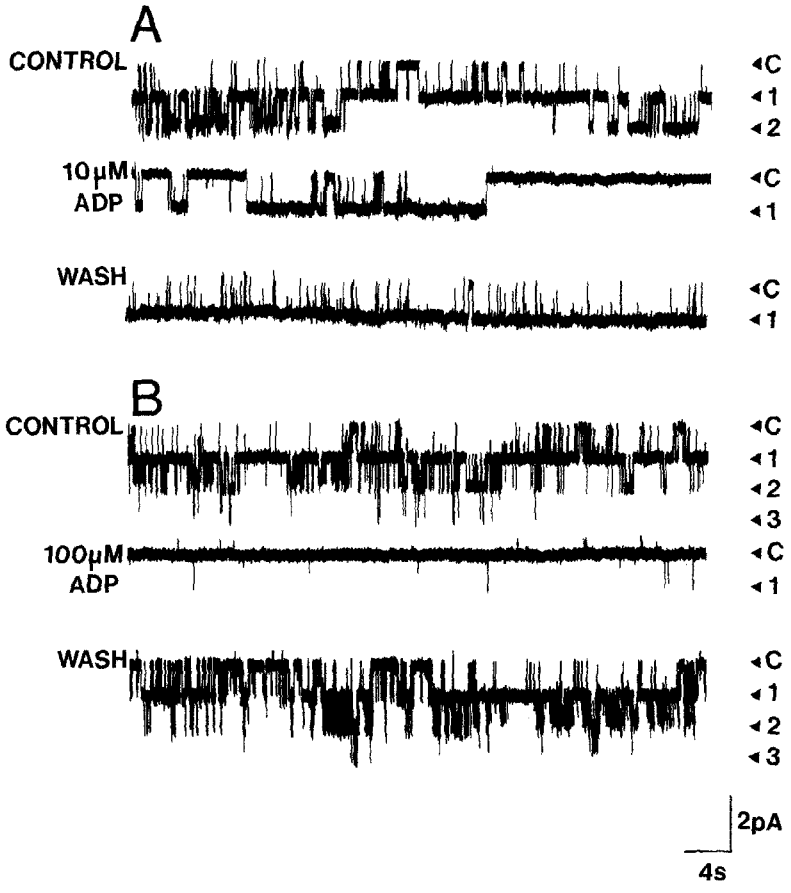


**Fig. 2.** Single channel current records showing the inhibitory effects of different concentrations of ADP, 10 μM (*A*) and 100 μM (*B*) in two separate patches. Ca-NS$^+$ channel currents were recorded from excised inside-out patches held at a membrane potential of −45 mV in the presence of 0.5 mM $Ca^{2+}$. Single channel openings are denoted by downward deflections (inward currents). The values for $N_f \cdot P_o$ are as follows: (*A*) Control, 0.859; 10 μM ADP, 0.383; Wash, 0.905; (*B*) Control, 1.325; 100 μM ADP, 0.004; Wash, 1.195.

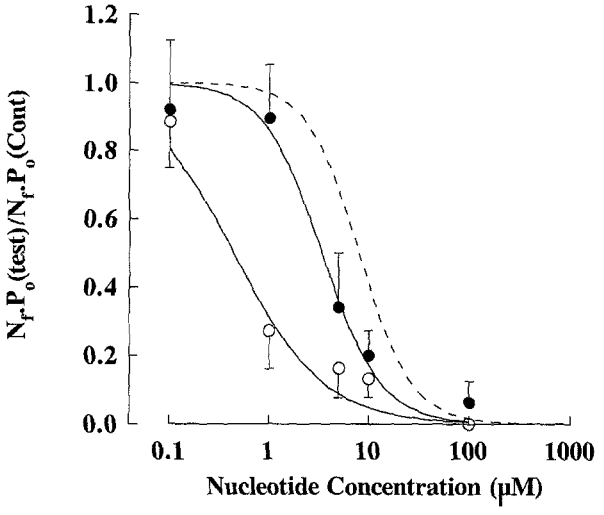


**Fig. 3.** The concentration dependence of the inhibitory effect of AMP (○) and ADP (●) on the single channel activity of the Ca-NS$^+$ channel measured in isolated inside-out patches held at a membrane potential of −45 mV in the presence of 0.5 mM $Ca^{2+}$. The values of $N_f \cdot P_o$ are plotted relative to the control value. In the absence of the nucleotides, this is 1.0. Values are expressed as the mean ± SEM of at least four patches. The smooth curves were fitted to the data using Eq. (1), according to a Marquardt-Levenberg least squares method (SigmaPlot Version 4.1). The relative inhibitory effect of ATP (dashed line) is shown for comparison using data from Sturgess et al. (1987).

degree of inhibition or no effect was seen on channel activity, and even after a 5 min exposure to cyclic AMP no increase in $N_f \cdot P_o$ occurred. The increase in channel activity induced by cyclic AMP was due to an increase in both $P_o$ and $N_f$.

At higher concentrations of cyclic AMP (≥10 μM), the activating effect of cyclic AMP declined and channel activity was reversibly reduced in the majority of patches. Upon exposure to concentrations of 10 or 100 μM cyclic AMP, 5 out of 18 patches exhibited an increase in Ca-NS$^+$ channel activity, the others only closure, and at a concentration of 1 mM cyclic AMP, 8 out of 8 patches showed inhibition. Furthermore, in patches where cyclic AMP induced channel activation at low concentrations, increasing the concentration of the cyclic AMP always resulted in channel closure (Fig. 5*B*). The inhibition of Ca-NS$^+$ channel activity by high concentrations of cyclic AMP was also associated with a reduction of both $N_f$ (channels entered a long-lived closed state, for as long as the cyclic AMP was present) and $P_o$. On washout of the cyclic nucleotide, there was an immediate return in $P_o$, but $N_f$ often remained suppressed. For example, in a single experiment where two channels were active in control, with an average $P_o$ of 0.46, 100 μM cyclic AMP reduced $P_o$ to 0.01 with one of the channels entering a long-lived closed state. On washout

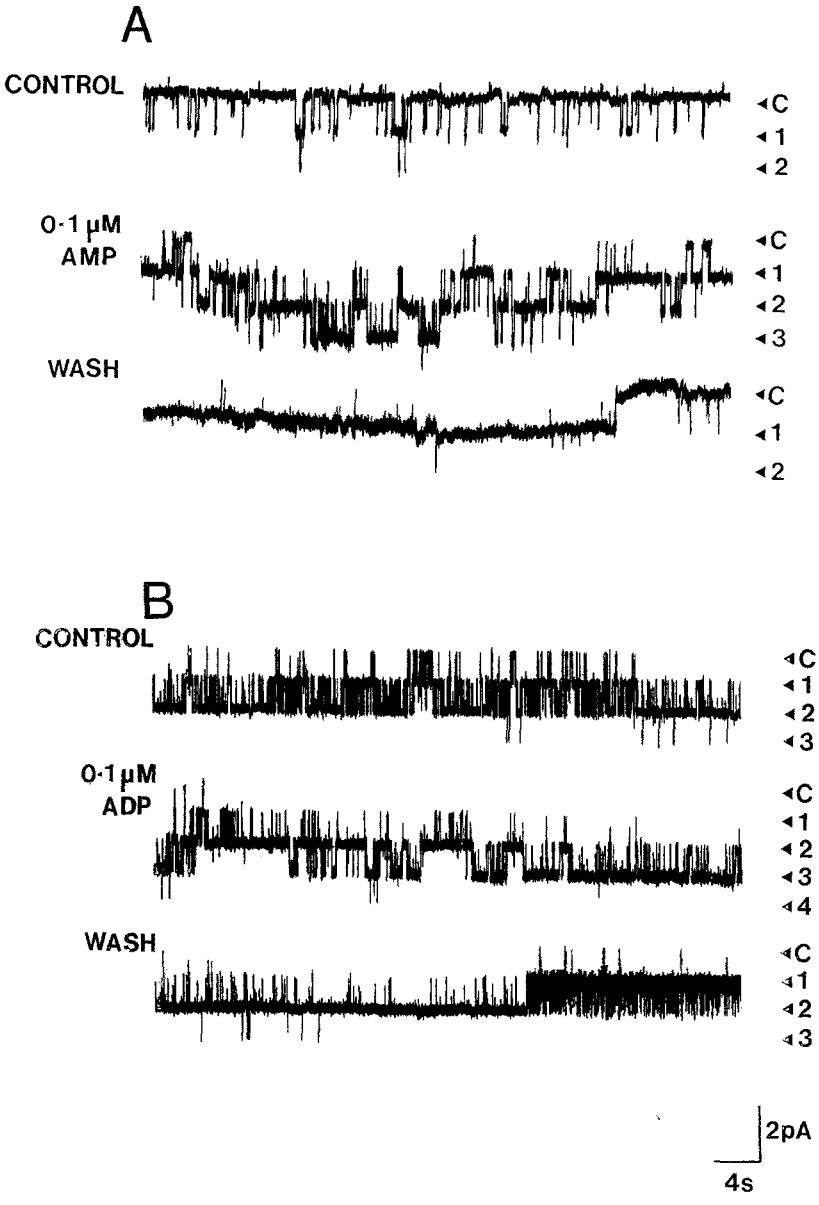


**Fig. 4.** (*A*) Single channel current records showing the activating effects of 0.1 μM AMP. Ca-NS$^+$ channel currents were recorded from excised inside-out patches held at a membrane potential of −45 mV in the presence of 0.2 mM $Ca^{2+}$. Single channel openings are denoted by downward deflections (inward currents). The values for $N_f \cdot P_o$ are as follows: Control, 0.062; 0.1 μM AMP, 1.609; Wash, 0.090. (*B*) Single channel current records showing the activating effects of 0.1 μM ADP. Ca-NS$^+$ channel currents were recorded from excised inside-out patches held at a membrane potential of −45 mV in the presence of 0.5 mM $Ca^{2+}$. Single channel openings are denoted by downward deflections (inward currents). The values for $N_f \cdot P_o$ are as follows: Control, 1.450; 0.1 μM ADP, 1.913; Wash, 1.600.

**Table 1.** Nucleotide specificity of Ca-NS$^+$ channel inhibition by monophosphorylated nucleotides

| Experimental condition (in 0.5 mM $Ca^{2+}$) | | $N_f \cdot P_o$(test)/ $N_f \cdot P_o$(control) | (*n*) |
|---|---|---|---|
| AMP | 0.1 mM | 0 | (5) |
| UMP | 1 mM | 0.24 ± 0.11 | (4) |
| CMP | 1 mM | 0.58 ± 0.09 | (4) |
| GMP | 1 mM | 0.67 ± 0.05 | (4) |

The results are expressed as the mean relative change from control in $N_f \cdot P_o$ ± SEM. The number of patches (*n*) used are shown in parentheses. A Student's *t*-test of the above data reveals that the values for UMP and GMP are significantly different at the 0.05 level ($P$ = 0.014) and the values for UMP and CMP are significantly different at the 0.05 level ($P$ = 0.05), while values for GMP and CMP are not significantly different at the 0.05 level ($P$ = 0.41).

of the cyclic AMP, $P_o$ increased to 0.3 but only one channel was active. Thus, the effects of cyclic AMP on Ca-NS$^+$ channel activity are concentration dependent, and a plot of the average activity ($N_f \cdot P_o$) in the presence of nucleotide (Fig. 6*A*) for all patches where cyclic AMP was tested ($n$ = 37) clearly indicates the biphasic nature of the action of this nucleotide. However, if the patches where cyclic AMP induced activation are excluded from the average and plotted separately from the inhibition data (Fig. 6*B*), it can be seen that, unlike the clear concentration-dependent inhibition induced by concentrations of cyclic AMP greater than 1 μM, there is no obvious concentration dependence for activation. The calculated half-maximal concentration of cyclic AMP for inhibition of Ca-NS$^+$ activity was 12 μM and had a Hill coefficient of 0.45.

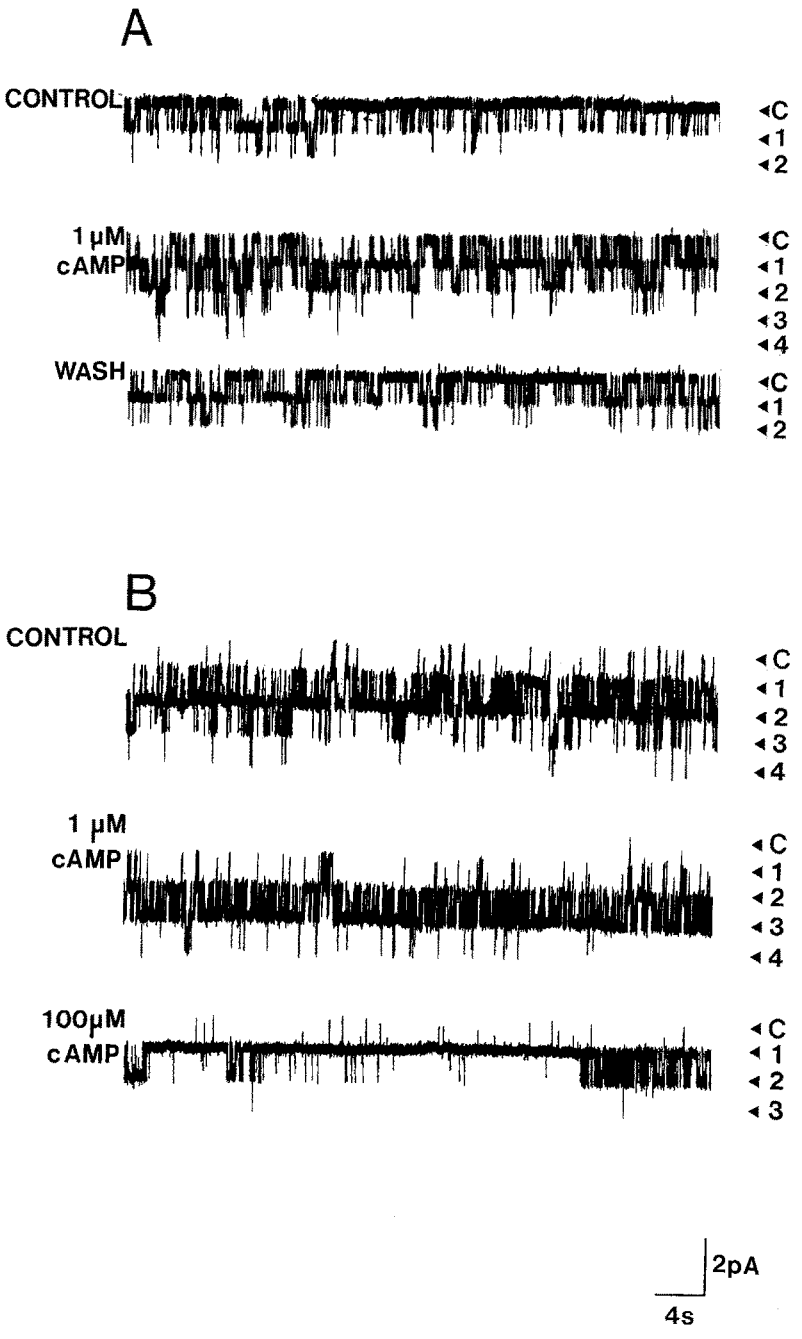


**Fig. 5.** (*A*) Single channel current records showing the activating effect of 1 μM cyclic AMP Ca-NS$^+$ channels currents were recorded from an excised inside-out patch in the presence of 0.2 mM $Ca^{2+}$. Single channel openings are denoted by downward deflections (inward currents) and the membrane potential was maintained at −45 mV. The values for $N_f \cdot P_o$ are as follows: Control, 0.151; 1 μM cyclic AMP, 0.329; Wash, 0.250. (*B*) Single channel current records showing the activating effect of 1 μM cyclic AMP and the inhibitory effect of 100 μM cyclic AMP on the activity of the Ca-NS$^+$ channel recorded from the same inside-out patch held at a membrane potential of −45 mV in the presence of 0.2 mM $Ca^{2+}$. The values of the $N_f \cdot P_o$ are as follows: Control, 1.848; 1 μM cyclic AMP, 2.438; and 100 μM cyclic AMP, 1.360. The nucleotide effects shown were fully reversible between the second and third traces and after the third trace (*not shown*) when the patch was washed with nucleotide-free solution.

Other cyclic nucleotides produced similar effects. In general, activation of channel activity was observed at low concentrations in a proportion of patches, with little effect or inhibition of channel activity at high concentrations for all the cyclic nucleotides tested. For example, in 9 out of 17 patches cyclic GMP (0.01–10 μM) induced an increase in Ca-NS$^+$ channel activity (mean activation 1.99 ± 0.38 over control) and at higher concentrations (100 μM and 1 mM) a small degree of channel inhibition was observed (10 out of 11 patches). Activation of Ca-NS$^+$ channel activity also occurred with 100 μM cyclic UMP (three out of six; mean activation 1.78 ± 0.28), 0.1–1.0 μM cyclic CMP (three out of four patches; mean activation, 1.64 ± 0.25) and 0.1–1.0 μM cyclic IMP (three out of seven; mean activation 2.91 ± 0.9). Consequently, the cyclic nucleotide site on this channel that mediates activation does not display any significant degree of base specificity. This is not so for the cyclic nucleotide site that mediates channel inhibition. This site on the Ca-NS$^+$ showed a strong preference for cyclic AMP over cyclic GMP, with cyclic UMP being almost equally potent with cyclic AMP. Cyclic IMP and cyclic CMP were not active at this site (Table 2*A*,*B*).

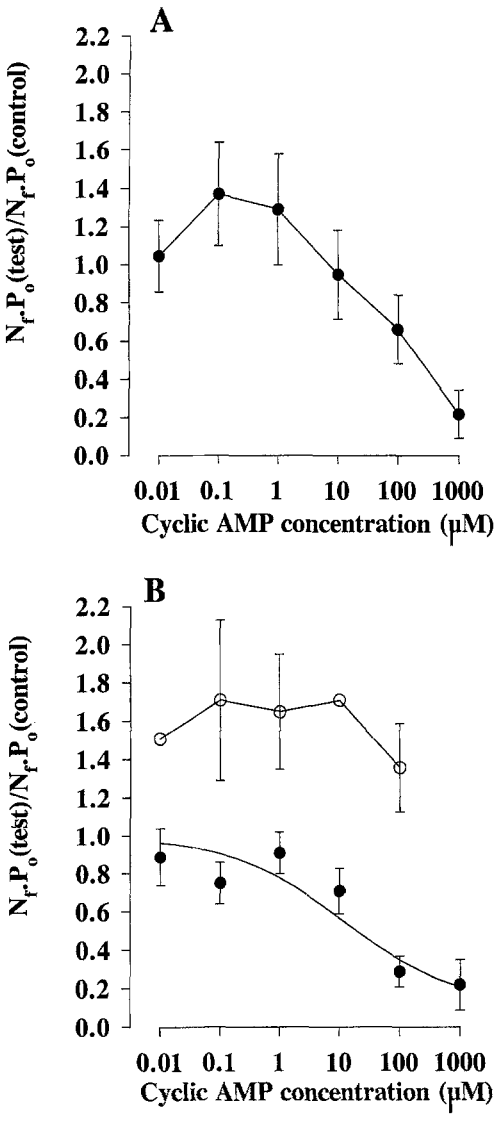


**Fig. 6.** Concentration dependence of the actions of cyclic AMP on single channel activity measured in isolated inside-out patches in the presence of 0.2 mM $Ca^{2+}$. Values of $N_f \cdot P_o$ are plotted relative to the control value (in the absence of cyclic AMP) prior to addition of the nucleotide. In the absence of cyclic AMP this is 1.0. (*A*) Average response to cyclic AMP of channel activity in all patches analyzed. Values are the means ± SEM of at least five patches. Note the variability in response to cyclic AMP at 0.1 and 1 μM. (*B*) Response to cyclic AMP in patches that exhibited distinct activations (○; mean ± SEM of at least three patches) in comparison to those that showed no effect or inhibition of channel activity (●; mean ± SEM of at least three patches). The data shown are a subset of *A*. The activation value at 0.01 μM is from only one patch and was 1.51, and for 10 μM the values are from two patches, 2.15 and 1.27.

### Cyclic AMP Activation Is Not Mediated by Protein Kinase

Cyclic AMP mediates many of its actions in intact cells through the activation of cyclic-AMP-dependent protein kinase activity. Therefore, one possible reason for the variability in observing activation of the Ca-$NS^+$ channel is the presence or absence (inactivation) of an endogenous kinase in the isolated inside-out patch. Figure 7 shows an experiment ($n = 3$) in which 1 μM cyclic AMP was applied to an inside-out patch producing an activation of channel activity to 3.2 over control. Following washout of the cyclic AMP and recovery of the channel activity, a second application of the same concentration of cyclic AMP in the presence of a protein kinase inhibitor (1 μM), induced a similar activation of channel activity (to 3.1 over control). The protein kinase inhibitor used in this experiment was a synthetic 20-residue peptide corresponding to the active sequence of a rabbit skeletal muscle protein kinase inhibitory protein (Cheng et al., 1986). A similar experiment was repeated using 10 μM ($n = 2$) of the protein kinase inhibitory peptide to try to block the activation of channel activity produced by 1 μM cyclic AMP. Again no inhibition of the cyclic AMP activation was observed suggesting the actions of cyclic AMP are likely to be through a direct interaction with the Ca-$NS^+$ channel.

**Table 2.** Nucleotide specificity of Ca-$NS^+$ channel inhibition by cyclic nucleotides

| Experimental condition | $N_f \cdot P_o$(test)/ $N_f \cdot P_o$(control) | (*n*) |
|---|---|---|
| (A) (0.1 mM) | | |
| Cyclic AMP | 0.29 ± 0.08 | (7) |
| Cyclic UMP | 0.44 ± 0.15 | (3) |
| Cyclic GMP | 0.61 ± 0.21 | (6) |
| Cyclic CMP | 0.92 ± 0.18 | (3) |
| Cyclic IMP | 0.99 ± 0.07 | (3) |
| (B) (1 mM) | | |
| Cyclic AMP | 0.22 ± 0.13 | (8) |
| Cyclic UMP | 0.26 ± 0.11 | (3) |
| Cyclic GMP | 0.83 ± 0.28 | (4) |

The results are expressed as the mean relative change from control in $N_f \cdot P_o$ ± SEM. The number of patches (*n*) used are shown in parentheses. A Student's *t*-test of the above data reveals that in *A*, the values for Cyclic AMP and Cyclic UMP are not significantly different at the 0.05 level ($P = 0.388$), whereas the values for Cyclic AMP and Cyclic GMP are significantly different at the 0.1 level ($P = 0.08$). The values for Cyclic AMP and Cyclic CMP and those for Cyclic AMP and Cyclic IMP are significantly different at the 0.05 level ($P = 0.0068$ and $P = 0.0012$, respectively). Similarly, in *B*, the values for Cyclic AMP and Cyclic UMP are not significantly different at the 0.05 level ($P = 0.866$), whereas those for Cyclic AMP and Cyclic GMP, and those for Cyclic UMP and GMP, are significantly different at the 0.05 level ($P = 0.046$ and $P = 0.0047$, respectively).

## Discussion

The open state probability of the Ca-$NS^+$ channel in this cell line is reduced by ATP in a concentration-dependent way (Sturgess et al., 1986*b*, 1987). The present

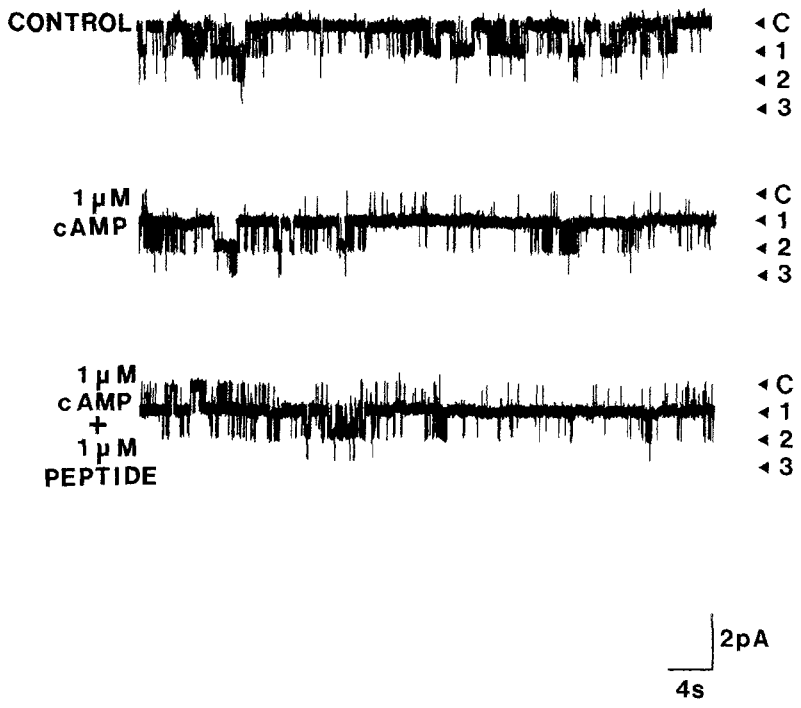


**Fig. 7.** Single channel current records to illustrate the activating effect of 1 μM cyclic AMP in the presence of 1 μM cyclic-AMP-dependent protein kinase inhibitory peptide. The control trace shows channel activity before exposure to 1 μM cyclic AMP alone (second trace). The patch was washed (*not shown*), and then exposed to a combination of 1 μM cyclic AMP plus 1 μM of the synthetic inhibitory peptide. Channel currents were recorded from an excised inside-out patch held at a membrane potential of −45 mV in the presence of 0.2 mM $Ca^{2+}$. $N_f \cdot P_o$ values are: Control, 0.308; 1 μM cyclic AMP, 0.979; and 1 μM cyclic AMP plus 1 μM synthetic inhibitory peptide, 0.943.

investigation has shown that the Ca-$NS^+$ channel activity is also reduced by AMP and ADP in a concentration-dependent manner, confirming earlier preliminary observations (Sturgess et al., 1986*b*). The present results are consistent with the suggestion (Sturgess et al., 1986*b*) that AMP is more potent than ADP and ATP at closing these channels. A similar order of potency has been reported for adenine-containing nucleotides causing inhibition of Ca-$NS^+$ channel activity in kidney cells (Paulais & Teulon, 1989). Ca-$NS^+$ channel activity is also blocked by ATP and AMP in a cultured secretory epithelial cell line (Cook, Poronnik & Young, 1990) and by ATP and ADP in mouse pancreatic acinar cells (Thorn & Petersen, 1992). The greater potency of AMP and ADP over ATP for the Ca-$NS^+$ channel in CRI-G1 cells suggests some similarity with $P_1$ purinergic receptors (Burnstock, 1978), but the much reduced effectiveness of adenosine (Sturgess et al., 1986*b*) is not compatible with this. The nucleotide specificity of the Ca-$NS^+$ channel is also very different from that of the ATP-sensitive $K^+$ ($K_{ATP}$) channel which is also present in this (Sturgess et al., 1986*a*) and other insulin-secreting cell lines (Dunne & Petersen, 1986*a,b*) and in isolated β-cells (Cook & Hales, 1984; Misler et al., 1986). ATP is much more effective than ADP and this channel is virtually insensitive to AMP and to adenosine suggesting a similarity with the $P_2$ purinergic receptor (Kakei, Noma & Shibasaki, 1985).

The observation that both ADP and AMP can activate the Ca-$NS^+$ channel in some patches, especially at low $Ca^{2+}$ concentrations, suggests that these nucleotides may modulate Ca-$NS^+$ channel activity by binding to multiple sites on the channel, one capable of increasing channel activity, the other of decreasing it. The increased variability of the response of the Ca-$NS^+$ channel to low concentrations of AMP and ADP (in the range 0.1–10 μM) could be provided by the relative degrees of activation and inhibition exhibited by the channels in individual patches. In contrast to the observations of Bokvist et al. (1991) on the $K_{ATP}$ channel, we have no evidence for time-dependent changes in the response of the Ca-$NS^+$ channel to the application of any of the nucleotides tested in the present study. As initially observed by Sturgess et al. (1986*b*), the responses of the Ca-$NS^+$ channel to adenine nucleotides (and in the present study to other nucleotides as well) was immediate and completely reversible by washing and could be reproduced many times with the same patch during prolonged recording periods of up to 2–3 hr. At present, the factors controlling this switch in responsiveness of the Ca-$NS^+$ channel to adenine-containing nucleotides in the CRI-G1 cells are not known. The transient channel activation that follows ATP removal has also been suggested to indicate a second nucleotide binding site on the Ca-$NS^+$ channel in pancreatic acinar cells (Thorn & Petersen, 1992). Nucleotides have also been suggested to modulate the activity of the $K_{ATP}$ channel in β-cells by binding to multiple sites (Dunne et al., 1988; Ashcroft & Rorsman, 1991; Hopkins et al., 1992). It is likely that there is one inhibitory site where both ATP and ADP act, and two separate activating sites, one for ADP and a second activated by ATP through a phosphorylation which also reduces channel "rundown" (Bokvist et al., 1991; Tung & Kurachi, 1991). It has

been suggested that in the intact β-cell, the ATP/ADP ratio is the important parameter in deciding the characteristics of the response of the $K_{ATP}$ channel to nucleotides (Dunne et al., 1988). However, the effects of different nucleotide ratios on the Ca-$NS^+$ channel have not yet been investigated.

Our results indicate that closure of the Ca-$NS^+$ channel is selective for adenine-containing nucleotides since AMP is much more potent than UMP or GMP; this is consistent with the findings of an earlier report (Sturgess et al., 1986*b*). Similar results were also obtained for the Ca-$NS^+$ channel from mouse kidney cells (Paulais & Teulon, 1989). $K_{ATP}$ channels have also been demonstrated to show some degree of nucleotide base specificity. The $K_{ATP}$ channel of frog skeletal muscle exhibits a high specificity for adenine-containing nucleotides since base modifications using other purines (GTP or ITP) or pyrimidines (CTP or UTP) reduced their effectiveness by about tenfold (Spruce, Standen & Stanfield, 1985, 1986, 1987). In ventricular myocytes, GTP and UTP produce a partial block of $K_{ATP}$ channel activity (Lederer & Nichols, 1984) and the $K_{ATP}$ of pancreatic β-cells is activated by low concentrations of GTP and GDP (0.01–1 mM) (Dunne & Petersen, 1986*a,b*) and inhibited by higher concentrations (*see* Ashcroft, 1988).

A novel finding of the present investigation is that the Ca-$NS^+$ channel in CRI-G1 cells is also sensitive to cyclic nucleotides. Cyclic AMP exhibits a dual effect, predominantly increasing channel activity at low concentrations (0.1–10 μM) and reducing it at higher concentrations (100 μM and 1 mM). A similar inhibitory effect of cyclic AMP at high concentrations has been shown for the Ca-$NS^+$ channel in kidney cells (Paulais & Teulon, 1989), although the authors of this report did not indicate the effects of low concentrations. By contrast, a direct activation of a nonselective cation conductance, the $i_f$ current, by cyclic AMP occurs in inside-out patches from the membrane of isolated rabbit sinoatrial node myocytes (cardiac pacemaker cells) at all concentrations tested from 0.01–100 μM (DiFrancesco & Tortora, 1991). Cyclic AMP also directly activates nonselective cation channels underlying sensory transduction in olfactory neurons (Nakamura & Gold, 1987) and in rod outer segments (Furman & Tanaka, 1989).

The actions of cyclic AMP on the Ca-$NS^+$ channel are likely to be direct and not via the actions of a cyclic-AMP-dependent protein kinase. This conclusion is supported by the observations that cyclic AMP modulates channel activity in patches in the absence of added ATP, and that the potent synthetic peptide inhibitor of cyclic-AMP-dependent protein kinase (Cheng et al., 1986) does not prevent cyclic-AMP-induced activation.

The cyclic nucleotide site on the CRI-G1 Ca-$NS^+$ channel mediating the inhibition of channel activity also exhibits base specificity, with cyclic AMP = cyclic UMP > cyclic GMP > cyclic CMP = cyclic IMP. It has also been reported that cyclic AMP is much more potent than cyclic GMP in inhibiting a nonselective cation channel in kidney cells (Paulais & Teulon, 1989) and in cardiac pacemaker cells (DiFrancesco & Tortora, 1991), with cyclic CMP having a significant, but lower effect than the other two nucleotides in the latter case. The second site on the CRI-G1 Ca-$NS^+$ channel mediating the increase in channel activity shows much less nucleotide specificity, with cyclic AMP, cyclic GMP and cyclic IMP being almost equally active at this site. Similarly, the nonselective cation channel in olfactory membrane patches showed an almost equal affinity for cyclic AMP and cyclic GMP ($K_{1/2}$ around 2 μM for both), with cyclic CMP being about 20- to 30-fold less potent in excised patches from olfactory neurons (Nakamura & Gold, 1987). However, it should be noted that when this channel was cloned from a rat olfactory cDNA library and expressed transiently in human embryonic kidney cells, it showed a 30-fold preference for cyclic GMP over cyclic AMP (Dhallan et al., 1990). The cyclic-nucleotide-gated channel in rods and cones was originally described to be highly selective for cyclic GMP, and not to be activated by cyclic AMP at concentrations up to 1 mM (Fesenko, Kolesnikov & Lyubarsky, 1985; Haynes & Yau, 1985). Nonetheless, more recently cyclic AMP has been reported to be a partial agonist of photoreceptor nonselective cation channels, activating only a fraction of the maximal cyclic GMP current (Tanaka, Eccleston & Furman, 1989; Ildefonse, Crouzy & Bennett, 1992), but being capable of potentiating the current produced by low doses of cyclic GMP (Filatov et al., 1989; Furman & Tanaka, 1989). The cyclic-GMP-dependent conductance of the fragments from frog rod plasma membranes has also been suggested to be modulated by ATP, GTP and cyclic AMP at a number of as yet undefined regulatory sites different from the site of action of cyclic GMP (Filatov et al., 1989).

Thus, it seems that the Ca-$NS^+$ channel in the CRI-G1 cells may have at least two different cyclic nucleotide regulatory sites, in addition to the two sites described above for noncyclic nucleotides. In the absence of specific blocking agents for these sites, the degree of cross-reactivity of each of the nucleotides is difficult to predict.

The exact physiological role of the Ca-$NS^+$ channel in the intact cells of the insulin-secreting cell line CRI-G1 is not known. In addition, its presence has not yet been determined in cell-attached patches from normal β-cells, although Ca-$NS^+$ channel-like activity could be recorded in excised inside-out patches from these cells (Reale, 1992). However, the channel is present in CRI-G1 cells at a density equal to that of the ATP-$K^+$ channel (Sturgess et al., 1986*a,b*, 1987) and since its open state probability depends on a complex in-

terrelationship between intracellular calcium concentration, membrane voltage and the intracellular concentrations of adenine-containing nucleotides, it could well be in a unique position to modulate the process of insulin release. For instance, the glucose-induced closure of the ATP-$K^+$ channels in β-cells (Ashcroft, 1988; Cook et al., 1988) will only cause a depolarization in the presence of a background inward current. Thus, at least a part of the sodium-dependent background inward current that underlies the glucose-evoked depolarization in the rat insulinoma cell line RINm5F (Dunne et al., 1989, 1990) could be due to the activity of the Ca-$NS^+$ channel. Indeed, the sensitivity of the Ca-$NS^+$ channel to cyclic nucleotides opens up the possibility that it could be involved in the modulation of the glucose-induced insulin release by agents such as glucagon, somatostatin and galanin, which alter cyclic nucleotide levels in the β-cells (Prentki & Matschinsky, 1987; De Weille et al., 1989).

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## References

Ashcroft, F.M. 1988. Adenosine 5′-triphosphate-sensitive potassium channels. *Annu. Rev. Neurosci.* **11:**97–118

Ashcroft, F.M., Rorsman, P. 1991. Electrophysiology of the pancreatic β-cell. *Prog. Biophys. Mol. Biol.* **54:**87–143

Bevan, S., Gray, P.T.A., Ritchie, J.M. 1984. A calcium-activated cation-selective channel in rat cultured Schwann cells. *Proc. R. Soc. (Lond.) B.* **222:**349–355

Bokvist, K., Ämmälä, C., Ashcroft, F.M., Berggren, P.-O., Larsson, O., Rorsman, P. 1991. Separate processes mediate nucleotide-induced inhibition and stimulation of the ATP-regulated $K^+$-channels in mouse pancreatic β-cells. *Proc. R. Soc. (Lond.) B.* **243:**139–144

Burnstock, G. 1978. A basis of distinguishing two types of purinergic receptor. *In:* Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach. L. Bolis and R.W. Straub, editors. pp. 107–118. Raven, New York

Carrington, C.A., Rubery, E.D., Pearson, E.C., Hales, C.N. 1986. Five new insulin-producing cell lines with differing secretory properties. *J. Endocrinol.* **109:**193–200

Cheng, H.-C., Kemp, B.E., Pearson, R.B., Smith, A.J., Misconi, L., Van Patten, S.M., Walsh, D.A. 1986. A potent synthetic peptide inhibitor of the cAMP-dependent protein kinase. *J. Biol. Chem.* **261:**989–992

Colquhoun, D., Neher, E., Reuter, H., Stevens, C.F. 1981. Inward current channels activated by intracellular $Ca^{2+}$ in cultured cardiac cells. *Nature* **294:**752–754

Cook, D.L., Hales, C.N. 1984. Intracellular ATP directly blocks $K^+$ channels in pancreatic β-cells. *Nature* **311:**271–273

Cook, D.I., Poronnik, P., Young, J.A. 1990. Characterization of a 25-pS nonselective cation channel in a cultured secretory epithelial cell line. *J. Membrane Biol.* **114:**37–52

Cook, D.L., Satin, L.S., Ashford, M.L.J., Hales, C.N. 1988. ATP-sensitive $K^+$ channels in pancreatic β-cells. Spare-channel hypothesis. *Diabetes* **37:**495–498

Dempster, J. 1988. Computer analysis of electrophysiological signals. *In:* Microcomputers in Physiology: A Practical Approach. P.J. Fraser, editor. pp. 51–92. IRL, Oxford

De Weille, J.R., Schmid-Antomarchi, H., Fosset, M., Lazdunski, M. 1989. Regulation of ATP-sensitive $K^+$ channels in insulinoma cells: Activation by somatostatin and protein kinase C and the role of cAMP. *Proc. Natl. Acad. Sci. USA* **86:**2971–2975

Dhallan, R.S., Yau, K.-W., Schrader, K.A., Reed, R.R. 1990. Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. *Nature* **347:**184–187

DiFrancesco, D., Tortora, P. 1991. Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. *Nature* **351:**145–147

Dunne, M.J., Gallacher, D.V., Petersen, O.H., Yule, D.I. 1989. $Na^+$-dependent glucose-evoked depolarization and increase in $[Ca^{2+}]_i$ in the rat insulinoma cell line RINm5F. *J. Physiol.* **415:**93*P*

Dunne, M.J., Petersen, O.H. 1986*a*. GTP and GDP activation of $K^+$ channels that can be inhibited by ATP. *Pfluegers Arch.* **407:**564–565

Dunne, M.J., Petersen, O.H. 1986*b*. Intracellular ADP activates $K^+$ channels that are inhibited by ATP in an insulin-secreting cell line. *FEBS Lett.* **208:**59–62

Dunne, M.J., West-Jordan, J.A., Abraham, R.J., Edwards, R.H.T., Petersen, O.H. 1988. The gating of nucleotide-sensitive $K^+$-channels in insulin-secreting cells can be modulated by changes in the ratio $ATP^{4-}/ADP^{3-}$ and by nonhydrolyzable derivatives of both ATP and ADP. *J. Membrane Biol.* **104:**165–177

Dunne, M.J., Yule, D.I., Gallacher, D.V., Petersen, O.H. 1990. Stimulant-evoked depolarization and increase in $[Ca^{2+}]_i$ in insulin-secreting cells is dependent on external $Na^+$. *J. Membrane Biol.* **113:**131–138

Fesenko, E., Kolesnikov, S., Lyubarsky, A. 1985. Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature* **313:**310–313

Filatov, G.N., Jainazarov, A.B., Kolesnikov, S.S., Lyubarsky, A.L., Fesenko, E.E. 1989. The effect of ATP, GTP, and cAMP on the cGMP-dependent conductance of the fragments from frog rod plasma membrane. *FEBS Lett.* **245:**185–188

Furman, R.E., Tanaka, J.C. 1989. Photoreceptor channel activation: Interaction between cAMP and cGMP. *Biochemistry* **28:**2785–2788

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Haynes, L., Yau, K. 1985. Cyclic GMP-sensitive conductance in outer segment membrane of catfish cones. *Nature* **317:**61–64

Hille, B. 1984. Ionic Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA

Hockberger, P.E., Swandulla, D. 1987. Direct ion channel gating: a new function for intracellular messengers. *Cell. Mol. Neurobiol.* **7:**229–236

Hopkins, W.F., Fatherazi, S., Peter-Riesch, B., Corkey, B.E., Cook, D.L. 1992. Two sites for adenine-nucleotide regulation of ATP-sensitive potassium channels in mouse pancreatic β-cells and HIT cells. *J. Membrane Biol.* **129:**287–295

Ildefonse, M., Crouzy, S., Bennett, N. 1992. Gating of retinal rod cation channel by different nucleotides: comparative study of unitary currents. *J. Membrane Biol.* **130:**91–104

Kakei, M., Noma, A., Shibasaki, T. 1985. Properties of adenine-triphosphate-regulated potassium channels in guinea pig ventricular cells. *J. Physiol.* **363:**441–462

Kass, R.S., Lederer, W.J., Tsien, R.W., Weingart, R. 1978. Role of

calcium ions in transient inward currents and after contractions induced by strophantidin in cardiac Purkinje fibres. *J. Physiol.* **281:** 187–208

Kaupp, U.B. 1991. The cyclic nucleotide-gated channels of vertebrate photoreceptors and olfactory epithelium. *Trends. Neurosci.* **14:** 150–157

Kozlowski, R.Z., Ashford, M.L.J. 1990. ATP-sensitive $K^+$-channel run-down is $Mg^{2+}$ dependent. *Proc. R. Soc. (Lond.) B.* **240:**397–410

Kozlowski, R.Z., Hales, C.N., Ashford, M.L.J. 1989. Dual effects of diazoxide on ATP-$K^+$-currents recorded from an insulin-secreting cell line. *Br. J. Pharmacol.* **97:**1039–1050

Lederer, W.J., Nichols, C.G. 1989. Nucleotide modulation of the activity of rat heart ATP-sensitive $K^+$ channels in isolated membrane patches. *J. Physiol.* **419:**193–211

Maruyama, Y., Petersen, O.H. 1982. Cholecystokinin activation of single-channel currents is mediated by internal messenger in pancreatic acinar cells. *Nature* **299:**159–161

Maruyama, Y., Petersen, O.H. 1984. Single calcium-dependent cation channels in mouse pancreatic acinar cells. *J. Membrane Biol.* **81:** 83–87

Misler, S., Falke, L.C., Gillis, K., McDaniel, M.L. 1986. A metabolite-regulated potassium channel in rat pancreatic β-cells. *Proc. Natl. Acad. Sci. USA* **83:**7119–7123

Nakamura, T., Gold, G. 1987. A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature* **325:**442–444

Partridge, L.D., Swandulla, D. 1988. Calcium-activated non-specific cation channels. *Trends. Neurosci.* **11:**69–72

Paulais, M., Teulon, J. 1989. A cation channel in the thick ascending limb of Henle's loop of the mouse kidney: inhibition by adenine nucleotides. *J. Physiol.* **413:**315–327

Prentki, M., Matschinsky, F.M. 1987. $Ca^{2+}$, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol. Rev.* **67:**1185–1248

Reale, V. 1992. Nucleotide Modulation of Non-Selective Cation Channels in an Insulin-secreting Cell Line. Ph.D. Thesis, University of Cambridge

Reale, V., Hales, C.N., Ashford, M.L.J. 1992. Cyclic AMP regulates a calcium-activated non-selective cation channel in a rat insulinoma cell line. *J. Physiol.* **446:**312*P*

Spruce, A.E., Standen, N.B., Stanfield, P.R. 1985. Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* **316:**736–738

Spruce, A.E., Standen, N.B., Stanfield, P.R. 1986. The effect of nucleotides on the adenosine triphosphate-regulated potassium channel in frog skeletal muscle. *J. Physiol.* **373:**63*P*

Spruce, A.E., Standen, N.B., Stanfield, P.R. 1987. Studies of the unitary properties of adenosine-5′-triphosphate-regulated potassium channels of frog skeletal muscle. *J. Physiol.* **382:**213–236

Stoeckel, H., Takeda, K. 1989. Calcium-activated, voltage-dependent, non-selective cation currents in endosperm plasma membrane from higher plants. *Proc. R. Soc. (Lond.) B.* **237:**213–231

Sturgess, N.C., Ashford, M.L.J., Cook, D.L., Hales, C.N. 1986*a*. Single channel recordings of potassium currents in an insulin secreting cell line. *J. Endocrinol.* **109:**201–207

Sturgess, N.C., Hales, C.N., Ashford, M.L.J. 1986*b*. Inhibition of a calcium-activated non-selective cation channel, in a rat insulinoma cell line, by adenine derivatives. *FEBS Lett.* **208:**397–400

Sturgess, N.C., Hales, C.N., Ashford, M.L.J. 1987. Calcium and ATP regulate the activity of a non-selective cation channel in a rat insulinoma cell line. *Pfluegers Arch.* **409:**607–615

Swandulla, D., Partridge, L.D. 1990. Non-specific cation channels. *In:* Potassium Channels. Structure, Classification, Function and Therapeutical Potential. N.S. Cook, editor. pp. 167–180. Ellis Horwood, Chichester, UK

Tanaka, J.C., Eccleston, J.F., Furman, R.E. 1989. Photoreceptor channel activation by nucleotide derivatives. *Biochemistry* **28:**2776–2784

Thorn, P., Petersen, O.H. 1992. Activation of nonselective cation channels by physiological cholecystokinin concentrations in mouse pancreatic acinar cells. *J. Gen. Physiol.* **100:**11–25

Tung, R.T., Kurachi, Y. 1991. On the mechanism of nucleotide diphosphate activation of the ATP-sensitive $K^+$ channel in ventricular cell of guinea-pig. *J. Physiol.* **437:**239–256